ARTICLE

Genetics, Hormonal, and Genital Features of Congenital Adrenal Hyperplasia Patients In Semarang

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Abstract

Congenital Adrenal Hyperplasia (CAH) is a familial disorder derived from reduction activity of enzymes in steroidogenesis process of the adrenal cortex that caused by a mutation of CYP21 gene located at 6p21.3. Prominent manifestation of CAH patients is ambiguity on their genitalia.

This was a descriptive observational retrospective and prospective study following genetics, hormonal, and genital features from subjects with suspected CAH. Data was obtained using medical records from the Molecular and Cytogenetic Unit in Faculty of Medicine Diponegoro University and cytogenetic laboratory of Telogorejo hospital in Semarang since April 2004 to April 2005.

Of the 79 patients diagnosed as ambiguous genitalia and hypospadia, 17 patients were suspected CAH and three of them were with mental retardation. Mutation analysis of CYP21 gene was done for 11 (64,7%) patients. Of the 8 cases (72,7%) depicted common homozygous mutations (i.e. 163 bp del, IVS2-13A>G, K102R, S493N, I172N, R356W, S268T), 6 cases (75%) demonstrated other common heterozygote mutation. Interestingly, 3 patients had heterozygous mutations (i.e. one patient with heterozygote Q318X, one with heterozygote V281L+920-921insT+Q381X and the other had heterozygote mutations I172N + R356 W all in 1 allele) manifested as simple virilizing CAH. The most frequent mutations were S493N (20%) followed by IVS2-13A>G (15%) and I172N, R356W (14%) respectively. These frequencies are rather different than other published data in Caucasian population. Among those cases were found two familial cases, patients with suspect adrenal tumours, aromatase deficiency and glucocorticoid receptor defect. Only 8 patients (47%) had specific hormonal values for CAH. Clinical stigmata were varies, however all patients developed enlarged clitoris.

Establishing physical examination alone is not effective in diagnosing CAH. Cytogenetic, hormonal assay and mutation analysis are essential for assessments.

Keywords : *ambiguous genitalia, congenital adrenal hyperplasia, CAH, CYP21 gene*

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Abstrak

Kongenital Adrenal Hiperplasia (HAK) merupakan kelainan familial yang disebabkan oleh menurunnya aktivitas enzim yang berperan pada proses steroidogenesis di kelenjar adrenal oleh karena mutasi pada gen CYP21, terletak pada kromosom 6p21.3 yang mengkode enzim- enzim tersebut. Ambiguitas pada kelamin merupakan manifestasi utama pasien HAK.

Penelitian ini merupakan penelitian deskriptif observasional retrospektif dan prospektif tentang gambaran distribusi genetika, hormon dan keadaan kelamin pasien HAK. Data diambil dari catatan medik pada unit sitogenetik dan molekular Fakultas Kedokteran Universitas Diponegoro dan laboratorium sitogenetik rumah sakit Telogorejo Semarang periode April 2004 sampai April 2005.

Dari 79 pasien ambigus genitalia dan hipospadia, 17 diantaranya merupakan pasien HAK dan tiga (17,65%) dari mereka adalah pasien HAK dengan retardasi mental. Sekuensing DNA pada gen CYP21 dilakukan pada 11 (64,70%) pasien. Dari 8 (72,72%) pasien yang menggambarkan mutasi homozigot (163 bp del, IVS2-13A>G, K102R, S493N, I172N, R356W, S268T), enam diantaranya (75%) ternyata juga memiliki mutasi heterezigot lain. Hal yang menarik adalah ditemukannya 3 pasien yang memiliki mutasi heterozigot (satu pasien dengan mutasi heterozigot Q318X, satu pasien dengan mutasi heterozigot V281L+ 920-921insT +Q318X dan yang lain dengan mutasi heterozigot I172N dan R356W ketiganya pada 1 alel) yang menunjukkan HAK tipe virilisasi sederhana. Mutasi yang paling sering terjadi adalah mutasi pada S493N sebanyak 20% diikuti IVS2-13A>G sebanyak 15%, I172N dan R356W sebanyak 14%. Hasil ini sedikit berbeda dari data frekuensi mutasi pada orang Kaukasia. Kasus menarik yang lainnya adalah adanya 2 kasus HAK familial, pasien dengan kecurigaan tumor kelenjar adrenal, defisiensi aromatase, dan defek pada reseptor glukokortikoid. Delapan pasien (47,06%) memiliki gambaran hormon yang spesifik untuk HAK. Semua pasien menunjukkan pembesaran clitoris.
Pemeriksaan fisik saja tidak efektif untuk mendiagnosa HAK. Pemeriksaan sitogenetik, hormon, dan analisa mutasi penting sebagai pemeriksaan lanjut.

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Kata kunci : *ambigus genitalia, congenital adrenal hyperplasia, CAH, gen CYP21*

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Introduction

Congenital Adrenal Hyperplasia (CAH) is autosomal recessive disorder caused by reduced activity of enzymes required for steroidogenesis in the adrenal cortex.[1] This group of diseases is due to mutations in the genes encoding several enzymes needed for the production of adrenal cortex hormones.[2] CAH comprises the most frequent cause of ambiguous genitalia in the newborn, almost 60 % of all intersexes cases. It produces a female pseudohermaphroditism, a genital female with virilized phenotype.[3]

21-Hidroxylase deficiencies become the most common cause of CAH, 90% of all CAH cases. This occurs in two forms: classical and non-classical. The classical form has two types: Salt wasting (SW), and Simple Virilizing (SV) type. The second most common cause of CAH (5-8%) of all CAH cases is the 11-β hydroxylase enzyme deficiency. Less other common cause of CAH is deficiencies of the following enzyme: 3-Beta-hydroxysteroid dehydrogenase; Aldosterone synthase; 17-α hydroxylase deficiency; and mutations in the steroidogenic acute regulatory (StAR) protein.[4]

Clinical manifestation of CAH is different in male to that in female. Accelerated height, premature masculinization, and precocious sexual hair appear in childhood are features, that commonly found in male patients. While female patients, are more jeopardized by inappropriate sex assignment, making the genitalia may appear more masculine. The clitoris is enlarged resembling a small penis. The cleft between the labia may be partly closed over (fusion of the labia majorae) hiding the entrance to the vagina. Sometimes only one opening can be seen. [5,23] In classical CAH, there is prenatal virilization of external genitalia in females graded according to severity into Prader stages I-V. There is also progressive postnatal virilization in both sexes including accelerated growth and advancement of bone age during the first years of life lead to early epiphyseal closure. Non- classic, milder forms of CAH, includes precocious pubarche in children and acne, hirsutism, and menstrual irregularities in women.[17] Problems that follow patients with CAH are involving medical aspects as well as psychosexual aspects. Salt wasting nephropathy occurs in 75% of infants born with CAH. Several types of tumours (adrenal, testicular, and pituitary) tend to develop in some CAH patients. If it goes unrecognized, hypotension may happen and cause collapse. Some patients can be admitted to hospital for developing cerebral atrophy, spastic cerebral palsy and seizures. McGuire and Ommen in 1975 found indication that CAH patients do not have higher IQs than expected from family background. This is also similar to what Wenzel et al, 1978 discovered. There is even a case report about CAH patients with severe mental retardation. However, the correlation between mental retardation in CAH patients is still not well understood. [12]

Clinical manifestation, hormonal, and analysis of mutations on related genes encoding enzyme of steroidogenesis process are decisive for diagnosing CAH patients. In clinical data, we can see ambiguous genitalia as the main appearance of CAH cases primarily in female patients. Elevation of progesterone, 17-OH progesterone, and testosterone and decrease of cortisol and aldosterone are prominent hormonal features of CAH. Definite diagnosis of CAH is established by having cytogenetics and molecular analysis. A karyotype of 46, XX must be found on patients with CAH, since most CAH patients who come to doctor are usually female. Male cases are usually asymptomatic. [17]

CYP21 gene, located on short arm of chromosome 6 (6p21.3) is a cytochrome P450 enzyme located in the endoplasmic reticulum and which catalyzes the conversion of 17-hydroxyprogesterone to 11-deoxycortisol and progestene to deoxycorticosterone.[16] Mutations are related with deletion and gene conversion on CYP21 gene.[13, 15] There were many well known mutation on CYP21 related to CAH cases i.e 163 bp del, IVS2-13A>G, K102R, S493N, I 172N, R 356W, P 30L, Q 318X, R 102 K.[20,21,24]

Unfortunately the CAH data in Indonesia is scanty, probably because many of the patients and medical personnel are less aware to seek medical treatment making majority of CAH cases are under diagnosed and maltreated. Moreover most of the patients come from low socio-economic background that cannot afford the cost for examination and therapy. This has been worsening by the lack of facilities required for diagnosing CAH cases.

There are two genetics laboratories in Semarang, Cytogenetics Laboratory in Telogorejo Hospital and the Molecular and Cytogenetics laboratory in Faculty of Medicine. Since the establishment of those laboratories in 1999, the CAH cases increase progressively. Presence the sexual adjustment team of Dr. Kariadi hospital as referral hospital dealing with ambiguous genitalia in Central Java makes the diagnosis of CAH improved significantly.

This research aim is to know the distribution of genetics, hormonal, and physical features of CAH patients recorded in Cytogenetics laboratories in Semarang since April 2004 to April 2005.

Subjects and Methods

Subjects

Subjects for this research are all ambiguous genitalia patients suspected for CAH examined in two cytogenetics laboratories in Semarang. Target populations are all ambiguous genitalia patients diagnosed as CAH clinically, hormonally and genetically. This research was using a secondary data from medical records of CAH patients during period of April 2004- April 2005. Data was obtained from physical examination, hormonal assay, cytogenetic and mutation analysis. Some of hormonal measurement and mutation analysis could not be examined in Indonesia due to financial reason.

Methods

This was a descriptive-observational retrospective and prospective study research. This research has been conducted in two places: Cytogenetics and Molecular of Biotechnology Laboratory of Medical Faculty of Diponegoro University Semarang and Cytogenetics Laboratory of Telogorejo Hospital Semarang. The sampling collection was done in the period from April 2004 to April 2005 for ambiguous genitalia patients diagnosed as CAH clinically,hormonally, and genetically.

Patients suspected for CAH, then examined for further investigation. Physical examination specifically designs for CAH was done i.e. Quigley stage, Prader stage, urethrogenital swelling, phallus length, chordae, and presence of secondary sexual sign. Gender type, pedigree and photograph on genitalia were also recorded. Heparinized and EDTA peripheral blood was drawn for chromosome and DNA studies. Cytogenetic examination was performed using chromosome G banding procedure.[27]. The next step was to extract DNA from leucocytes using salting out method as elsewhere.[25,26] Subsequent DNA and plasma were sent to Erasmus Medical Centre for hormonal analysis and mutation analysis.

Data processing on physical measurement and result of cytogenetic analysis was analyzed with descriptive method. The result of data processing was reported in table and chart.

Results

79 patients diagnosed as ambiguous genitalia and hypospadia since April 2004 to April 2005, with CAH cases accounted 17 out of 79 ambiguous genitalia and hypospadia cases and three (17,65%) of them were CAH patients with mental retardation. Followings are their hormonal, mutation analysis and physical examinations.

Table 1. Overview of hormonal values mutation analysis and genital features conducted on 17 CAH patients .

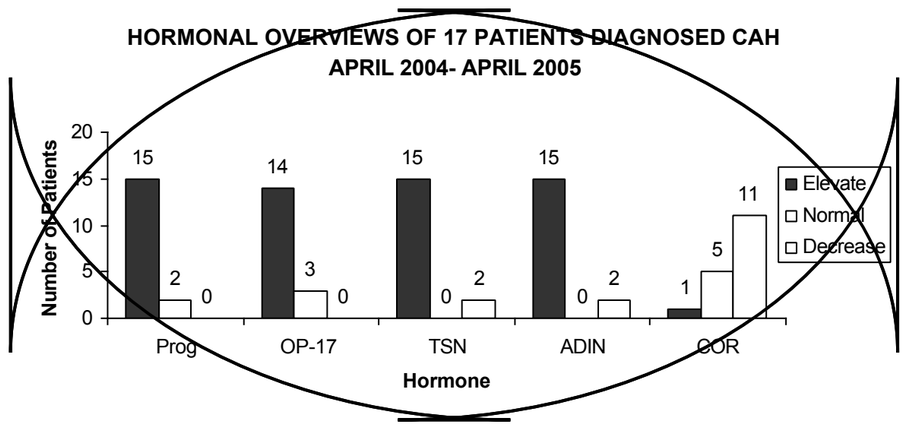
No	Age	PGN	OP17	TSN	ADIN	COR	Mutation		Gender	Q S	P S	Enlarged Clitoris(> 2cm)	Labio scrotal fusion
							Allele 1	Allele2					
1	33	N	N	↑	↑	↓	–	–	F	IV	II	+	Fusion labia minora
2	17	↑	↑	↑	↑	N	16 3bpdel IVS2-13A>G K102R S493N	16 3bpdel IVS2-13A>G K102R S493N	M	II	IV	+	Fusion labia majora
3	11	↑	↑	↑	↑	N	I172N S493N	I172N	F	V	I	+	Fusion labia minora
4	21	↑	N	↓	↓	↓	I172N R356W		F	>VI	normal female	+	No fusion
5	**11**	↑	↑	↑	↑	N	P30L R356W	R356W	F	III	III	+	Fusion labia majora
6	33	↑	↑	↑	↑	↓	I 172N R356W S493N	S493N	F	III	III	+	No fusion
7	6	↑	↑	↑	↑	N	Q318X		F	III	III	+	Labia majora fusion
8	**7**	↑	↑	↑	↑	↓	R356W	R356W	F	II	III	+	Fusion labia majora
9	7	↑	↑	↑	↑	↓	I172N	I172N	F	V	IV	+	Fusion labia minora

							S493N						
10	4	↑	↑	↑	↑	↓	IVS2-13A>G R102K S268T Q318X	IVS2-13A>G S268T	F	IV	I	+	Fusion labia minora
11	3	↑	↑	↑	↑	↓	16 3bpdel IVS2-13A>G K102R S493N	16 3bpdel IVS2-13A>G S493N	F	IV	II	+	No fusion
12	1.5	↑	↑	↑	↑	↓	–	–	F	III	II	+	Fusion labia majora
13	4w	↑	↑	↑	↑	↑	–	–	F	IV	NE	+	Fusion labia majora
14	8	N	N	↓	↓	↓	V281L 920-921insT Q318X		F	II	NE	+	Fusion labia majora
15	4w	↑	↑	↑	↑	N	–	–	F	III	NE	+	Fusion labia majora
16	1.9	↑	↑	↑	↑	↓	–	–	F	V	NE	+	Fusion labia majora
17	**10**	↑	↑	↑	↑	↓	–	–	F	IV	NE	+	No fusion

All cases were female gender with karotyping 46,XX except case no 2 were male with 46,XX karotyiping. Patients with bold fonts were CAH patients with mental retardation

↑: Increase ↓: Decrease N: normal QS : Quigley stage PS : Prader stage

Five different hormones were examined as parameters for CAH: Progesterone, 17-Hydroxyprogesterone, Testosterone, Androstenedione, and Cortisol.



Distribution of hormonal values of 17 patients diagnosed as CAH can be seen below.

Fig.1. Distribution of each hormonal measured.

Note:

Prog : Progesterone ADIN : Androstenedione

OP-17 P: 17- Hydroxy Progesterone COR : Cortisol

TSN : Testosterone

Hormonal analysis done in Rotterdam illustrated that 17 patients examined for progesterone hormone: 15 patients (88, 24%) showed elevation; 2 (11, 76%) were in normal values. For 17-hydroxyprogesterone: 14 patients (82,35%) showed elevation;

3 patients were in normal values (17,64%). 15 patients (88, 24%) showed elevation, with 2 of them (11, 76%) showed decrease for both testosterone and androstenedione hormone. For cortisol hormone: 1(5, 88%) showed elevation; 5 (29, 41%) were in normal values; 11 (64, 71%) showed decrease.

Increasing progesterone, 17-hydroxyprogesterone, testosterone, androstenedione and decreasing cortisol were found in 8 patients (47, 06%) from 17 suspected CAH. Aldosterone was not measured in this research.

Of 17 suspected CAH patients, 11 (64,70%) patients were analysed for CYP21 gene mutation. Common homozygous mutations (i.e. 163 bp del, IVS2-13A>G, K102R,

S 493N, I 172N, R 356W, S 268T) were found in 8 (72,72%) cases. Six (75%) of whom also demonstrated other heterozygote mutation (i.e. compound heterozygote for I172N+S 493N / I172N; P30L+R356W / R356W; I172N+R356W+S493N / S493N; I 172N + S493N / I 172N; IVS2-13A>G+R102K+S268T+Q318X / IVS2-13A>G+S 268T; 163 bp del+ IVS2-13A>G+K102R+S493N/163 bp del +IVS2-13A>G+S493N). Of 8 patients (47,06%) demonstrated specific hormonal values for CAH, 5 (29,41%) had confirmed mutation on CYP21 gene. Interestingly, 3 patients depicted heterozygous mutations (i.e. one patient with heterozygote mutation Q318X, one with heterozygote mutations V281L+920-921insT+Q318X and the other had heterozygote mutations I172N + R356W all in 1 allele) manifested as simple virilized CAH. The most frequent mutation was S493N (20%) followed by IVS2-13A>G (15%), I172N and R356W (14%) cases respectively. Distributions of mutations are as described below.

Fig.2. Distribution of mutations in CAH patients.

This study revealed variations of clinical features from CAH patients primarily on their genitalia. Most of them had stage III for Quigley (35, 29%) and stage II for Prader (23, 53%). 64,71% of patients had labia majora and minora, 29,41% had only labia major and 5,88% had scrotum bifidum. All of patients (100%) demonstrated enlarged clitoris more than 2 cm in length, particular sign for CAH. Below are results for physical examination.

Table 2. Results of physical examination in 17 CAH patients from April 2004- April 2005.

Clinical Examination	Classification	Total Patients	Percentage
Gender	Female	16	94,12%
	Male	1	5,88%
Quigley Stage	I	0	0%
	II	2	11,76%
	III	6	35,29%
	IV	5	29,41%
	V	3	17,65%
	>V	1	5,88%
Prader Stage	Normal Female	1	5,88%
	I	2	11,76%
	II	4	23,53%
	III	3	17,65%
	IV	1	5,88%
	V	0	0%
	NE (Not Examined)	6	35,29%
Labia Majora and Minora	Has Both	11	64,71%
	Only Labia Majora	5	29,41%
	Scrotum Bifidum with fusion labia majorae	1	5,88%
Labio fusion	Fusion labia majora	9	52,94%
	Fusion labia minora	4	23,53%
	Has no fusion	4	23,53%
Clitoris	Enlarge (> 2 cm)	17	100%

Chordae	Negative (-) Positive (+)	16 1	94,12% 5,88%
Ending in perineal	Two endings with introitus vaginae Two endings with blind end vaginae One ending	11 1 5	64,71% 5,88% 29,41%
Secunder sex sign at very young age		3	17,65%
Mental Retardation		3	17,65%

Discussion

Only 8 patients with specific hormonal values for CAH while the rest of the patients showed variation in hormonal values, they might have increasing testosterone and androstenedione but they had normal level of progesterone and 17-hydroxyprogesterone and vice versa. Some patients (e.g. patient no 4 and 7) received Prednison or Dexamethasone therapy nevertheless showing different cortisol level.

Genetically, CAH patients express 46, XX karyotyping for female patients or 46, XY for male patients. In this research 16 out of 17 patients (94, 11%) suspected CAH showed karyotyping of 46, XX, which is apposite to their known gender, female. But there was 1 patient (5, 55%) whose known gender is male with 46, XX karyotyping.

There have been few reports regarding CYP21 gene mutations in Asian people. A study of 28 Singaporean revealed that the intron 2 mutation (IVS2-1A/C-G) was the most common mutation followed by I172N and R356W.[18] This result is consistent to what Usui et al, 2004 reported from 36 Japanese patients with CAH. [19] In contrast to the Caucasians, it was reported that the most common mutations in descending orders for British population are: large scale deletions/conversions, the intron 2 splice mutation, R357W, and I172N. [20] Netherlands as many other western European countries has the same pattern of CYP21 gene mutation. The patterns showed the most common mutations are deletion/conversion followed by I2G, I172N, and R356W[21]. It was speculated that R356W mutation seems to occur more commonly in the Asian patients and less in Caucasian.[19]. Surprisingly in this study, the missense mutation S493N became the most frequent mutation followed by IVS2-13A>G, I172N and R356W makes it slight different to the other published data. All of them result from gene conversion.

The salt wasting or simple virilizing forms of CAH result from IVS2-13A>G mutation, is reported to be the most prevalent mutation in the CYP21 gene among all ethnic groups. [7] In 1988, Higashi et al identified this mutation results from aberrant splicing of I 2(conversion) involving the nucleotide no 656 A/C→G. According to Miller et al, 1996 this mutation is located in intron 2, 13 bases from the splice acceptor site of exon 3. Either a C-to-G or A-to-G mutation at nucleotide 13 causes severe forms of 21-OH deficiency. White et al, 1994 mentioned that this mutation correspond to 22% of salt wasting cases, 25% of simple virilizing form, 12% of non classical cases.

The simple virilizing form of CAH is often related to I172N mutation where there was a substitution at codon 172 that results in substitution of Isoleucin into Asparagine. [8,13] It is a missense mutation (conversion) of CYP21 gene that only affected the structure, not the expression of the protein (Chiou et al, 1990) results in a defective enzyme (Amor et al, 1988). I172N mutation is related to wild type of 17-hydroxyprogesterone and Progesterone activity. [9, 10] According to Speiser et al, 1992 of 88 families with 21-OH deficiency 16% had this mutation, with 2% enzyme activity and a simple virilizing phenotype.

The R356W mutation is associated with the non-classical type of 21-OH deficiency, where Arginine (Arg) 356 is replaced by Tryptophan. It is a missense mutation (conversion) results in radical Amino acid substitution (Chiou et al, 1990).

In this research there was also found one heterozygous patient for mutation P30L, a substitution of Leucine for Proline at codon 30. P30L is also somewhat related to wild activity of 17-hydroxyprogesterone and Progesterone as much as 60% and about 30% of normal activity for Progesterone. This type of mutation is presumed to be potentially giving effects to the non-classical type of 21-OH deficiency (Tusie et al, 1991).

Glutamine is changed to stop codon in Q318X mutation. This may result in a completely non-functional enzyme due to premature termination of translation of the mRNA. A homozygous mutation for this gene results in salt wasting type of CAH, while compound heterozygous mutation in this gene is associated with simple virilizing form (Kharat et al, 2004). Dracopoulou-Vabouli et al, 2001 found that P30L mutation is happened in 21,4% in the non-classical form from 111 unrelated subjects in the Hellenic population.

163 bp del is best described as 3 base pairs deletion/insertion (Leu deletion/ insertion) downstream codon 9. As described in many literature insertion 3 base pairs CTG has no effect on the enzymatic activity, and it is assumed that such change is a non-functional mutation (Rodriques et al, 1987)

Speiser et al, 1988 detected a change in codon 281 from GTG, encoding Valine, to TTG, encoding Leucine in 9 non-classical 21-OH deficiency patients. White et al, 1994 mentioned that 34% of all non-classical type is due to this mutation. While Mornet et al, 1991 mentioned that the val-281-to-leu mutation accounts for 75 to 80% of non-classic 21-hydroxylase deficiency.

Other mutations found were K102R, S493N and S268T. Both K102R and S493N are non-functional mutation (Rodriques et al, 1987). While it can be inferred that S268T mutation is non-functional mutation, which does not cause CAH (Tusie-Luna et al, 1991)

Patient no 1, 33 year old girl was with virilization on her genitalia. Established hormonal measurement indicated normal level of progesterone, and 17-hydroxyprogesterone despite the increasing of testosterone, androstenedione and decreasing of cortisol. There was no confirmation on CYP21 gene mutation; this can be understood since she had normal 17-hydroxyprogesterone, signifying no presence of mutation. However there was an increasing testosterone, led to masculinization for her genitalia. It is highly possible that she is suspect for adrenal tumour. But further investigation should be assessed to confirm the diagnosis.

In three patients (patient no 4, 7, and 14), there was single heterozygote mutation but those patients manifested a simple virilized CAH. Patient no 4 was with heterozygote mutation I172+R356W; patient no 7 was with heterozygote mutation Q318X while patient no 14 was with heterozygote mutation V281L+920-921insT+Q318X. It is very remarkable since theoretically a single heterozygote mutation will not clinically manifest CAH (CAH is autosomal recessive disorder). It is very likely that in these patients have other mutation somewhere in the region that can cause these features. This was confirmed by the increasing of 17-hydroxyprogesterone (except for patient no 4 and 14, they showed normal level of 17-hydroxyprogesterone) indicating presence of mutation. Other factors should be considered as part of the explanation since in most genetic cases environmental factors play a tribute role for the phenotypic of patients. Therefore further investigation should be done to confirm this phenomenon.

Patient no 13, a month year old baby girl come with virilization on her genitalia. She depicted high level all measured hormones and remarkably high level of cortisol. There was no confirmation on CYP21 gene mutation. It is possible that she is suffered from glucocorticoid receptor defect.

Patient no 14, an 8-year-old girl with ambiguous genitalia, showing phenotype of CAH (clitoris enlarged- see fig. 3) but levels of 17-hydroxyprogesterone and androstenedione are relatively normal. This excludes a CYP21 block. But the cortisol level is relatively low. Testosterone is below detection level, despite this girl had no steroid therapy. These indicate to a block early in steroid biosynthesis, probably at the level of cholesterol side chain cleavage or the StAR protein. The other consideration on explaining this feature is that this girl might suffer from aromatase deficiency. However another molecular analysis for this patient and her father also done in Nijmegen, and the result is that she and her father shared same type of heterozygote mutation V281L + 920-921 ins T +Q318X. Her mutation is located at the exact same allele as her father. Molecularly she and her father are confirmed to be carrier CAH, but this does not explain such phenotype on the genitalia she demonstrated. Therefore it is possible that other mutations are present on the other areas of the gene.

Two familial CAH cases were also found. Patient no 3 (see fig.4) and no 9 were sisters with simple virilized of CAH. Both demonstrated the same compound heterozygote mutation of I 172N+ S493N / I72N. They were also having elevation of progesterone, 17-hydroxy progesterone, testosterone and androstenedione although patient no 3 was having relatively normal level of cortisol while her sister had low level of cortisol. The other familial CAH cases were patient no 2 and no 11. Patient no 2 seemed to show a more severe form of virilization. It is quite understandable since she (patient no.2) demonstrated more frequency of homozygote mutation compare to her sister. As the other CAH family case, these patients also had same pattern of elevation of progesterone, 17-hydroxy progesterone, testosterone and androstenedione despite patient no 2 was having relatively normal level of cortisol while her sister had low level of cortisol.

The exposure of androgen in both classical CAH and nonclassical 21-hydroxylase deficiencies may cause acelerated linear growth velocity and diminished final height in both males and females.[6] This is similar to 3 of our cases (patient no2, 4 and 6) that displayed an adult short stature. Patient no 2, 17 year old girl with severe virilization CAH was having final height 145,5 cm. Patient no 4 is 21 year old girl with simple virilization CAH was having final height of 150 cm. While patient no 6 is 33 year old girl also manifested simple virilization CAH with final height of 149,5 cm.

Many variations were found in the physical examination of patients, therefore physical features could not be used alone in establishing the diagnosis. Other examination such as hormonal assays and mutation analysis of CYP21 gene is needed. Conversely most patients were from low socio economic level that they could not afford the cost of laboratory examination and therapy as well.

A cytogenetics laboratory is necessary to perform the genotype of ambiguous genitalia patients, but this laboratory only available in 4 cities in Java island for whole archipelago with the population about 220 million. Patients from rural areas who may have ambiguous genitalia can be barely detected. It results in the diagnosis of CAH is neither yet well-established nor well detected and lead to incorrect treatment.

Issues about ambiguous genitalia and CAH in Indonesia up to now are less popular around medical personnel and even in the community. There was an increasing tendency of ambiguous genitalia patients seek for medical treatment during the progress of this research. Unfortunately, most of them came late, this made the further management and therapies more complicated. The delay of CAH patients admitted to hospital causes some of them develop more complex abnormalities such as seizures, cerebral atrophy, cerebral palsy, or mental retardation.

Thus, it is suggested to conduct other research to provide a better understanding about CAH, prevalence of CAH in Indonesia and give new insight into the underlying process.

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List of Abreviations

1. CAH : Congenital Adrenal Hyperplasia
2. StAR : Steroid Acute Regulatory Protein
3. bp : base pairs
4. del : deletions
5. IVS2 : interfering sequence 2
6. ins : insertion
7. A : nucleic acid code for adenine
8. G : nucleic acid code for guanine
9. I : amino acid code for isoleucine
10. K : amino acid code for lysine
11. L : amino acid code for leucine
12. N : amino acid code for asparagine
13. P : amino acid code for proline
14. Q : amino acid code for glutamine
15. R : amino acid code for arginine
16. S : amino acid code for serine
17. T : amino acid code for threonine
18. V : amino acid code for valine
19. W : amino acid code for tryptophan
20. X : stop codon (TAG or TAA)